201-15133

March 10, 2004

Administrator
US EPA
P.O. Box 1473
Merrifield, VA 22116
Attn: Chemical Right-to-Know Program

Dear Administrator:

On behalf of the member companies of the HPV Committee, the International Association of Color Manufacturers is pleased to submit the test plan and robust summaries for C.I. Acid Yellow 23 (FD&C Yellow 5). The IACM HPV Committee has chosen not to belong to the HPV Tracker System for submission of test plans and robust summaries. We are therefore submitting the test plan and accompanying robust summaries directly to EPA to make available to the public. A hard copy of this submission is available upon request. The EPA registration number for the IACM HPV Committee is

Please feel free to contact me with any questions or comments you might have concerning the submission (tadams@therobertsgroup.net or 202-331-2325).

Sincerely,

Timothy Adams, Ph.D.
Technical Contact Person for IACM HPV

201-15133A

Test Plan for
C.I. Acid Yellow 23
CAS No. 1934-21-0

Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

The International Association of Color Manufacturers/HPV Committee
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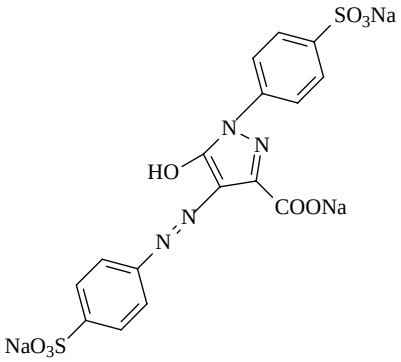
Test Plan for C.I. Acid Yellow 23

1 IDENTITY OF SUBSTANCES



C.I. Acid Yellow 23

CAS No. 1934-21-0

Synonyms:
FD&C Yellow No. 5
Tartrazine

2 CATEGORY ANALYSIS

2.1 INTRODUCTION

The International Association of Color Manufacturers (IACM) has volunteered to participate in the EPA's Chemical "Right-to-Know" Program. IACM is committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing on the chemicals used by the color industry in order to assure their human and environmental safety. The category analysis, test plan, and robust summaries presented represent the first phase of IACM's commitment to the Chemical "Right-to-Know" Program.

2.2 BACKGROUND INFORMATION

This category analysis and test plan provides data for FD&C Yellow No. 5. FD&C Yellow No. 5 is used as a food, drug, and cosmetic colorant. It is used to color candies and confections, bakery goods, cakes, cookies, ice cream, sherbets, cereals, soft drinks, sausage casings, jams and jellies, gelatin and pudding powders, beverage powders, maraschino cherries, prepared meats, canned and frozen vegetables, animal feeds, aqueous drug solutions, tablets, capsules, toothpastes, hair-waving fluids, bath salts, hair rinses, and printing inks for use in and on foods, drugs, and cosmetics and on food, drug, and cosmetic packaging materials.

FD&C Yellow No. 5 is an azo dye. Azo compounds are formed from arenediazonium ions reacting with highly reactive aromatic compounds, in what is called a diazo coupling reaction. Azo compounds are generally deeply colored because the azo linkage brings the two aromatic rings into conjugation [Solomon, 1996]. In addition to possessing extended conjugation, many azo dyes are also ring substituted with sulfonic acid substituents, which significantly increase polarity and water solubility and decrease absorption *in vivo*.

2.3 REGULATORY STATUS

FD&C Yellow No. 5 is a certified color additive approved in the United States to color food, drugs and cosmetics. Certified color additives are synthetic organic compounds that must meet high purity specifications established by the Food and Drug Administration (FDA) (see Table 1 below). Each batch of manufactured certified color in the United States is tested by the FDA for compliance with these specifications [Frick and Meggos, 1988]. Certified color additives are among the most thoroughly studied of all food ingredients because of the rigorous testing for human health endpoints required by the 1960 Color Additive Amendments to the FD&C Act [Hallagan, 1991]. There are currently only seven certified color additives approved for food, drug and cosmetic use in the United States.

Table 1. US FDA Specifications

FD&C Yellow No. 5 shall conform to the following specifications and shall be free from impurities other than those named to the extent that such other impurities may be avoided by good manufacturing practice (21 CFR 74.705):

• Sum of volatile matter at 135° C (275°F) and chlorides and sulfates (calculated as sodium salts), not more than 13 percent.

• Water-insoluble matter, not more than 0.2 percent.

• 4,4'-[4,5-Dihydro-5-oxo-4-[(sulfophenyl)hydrazono]-1H-pyrazol-1,3-diyl bis[benzenesulfonic acid], trisodium salt, not more than 1 percent.

• 4[(4',5-Disulfo[1,1'-biphenyl]-2-yl)hydrazono]]-4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole-3carboxylic acid, tetrasodium salt, not more than 1 percent.

• Ethyl or methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl) hydrazono] 1H-pyrazole-3-carboxylate, disodium salt, not more than 1 percent.

• Sum of 4,5-dihydro-5-oxo-1-phenyl-4-[(4-sulfophenyl)azo]-1H-pyrazole -3- carboxylic acid, disodium salt, and 4,5-dihydro-5-oxo-4-(phenylazo)-1-(4-sulfophenyl)-1H-pyrazole- 3- carboxylic acid, disodium salt, not more than 0.5 percent.

• 4-Aminobenzenesulfonic acid, sodium salt, not more than 0.2 percent.

• 4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole-3-carboxylic acid, disodium salt, not more than 0.2 percent.
• Ethyl or methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazole -3-carboxylate, sodium salt, not more than 0.1 percent.

• 4,4'-(1-Triazene-1,3-diyl)bis[benzenesulfonic acid], disodium salt, not more than 0.05 percent.
- 4-Aminoazobenzene, not more than 75 parts per billion.
- 4-Aminobiphenyl, not more than 5 parts per billion.
- Aniline, not more than 100 parts per billion.
- Azobenzene, not more than 40 parts per billion.
- Benzidine, not more than 1 part per billion.
- 1,3-Diphenyltriazene, not more than 40 parts per billion.
- Lead (as Pb), not more than 10 parts per million.
- Arsenic (as As), not more than 3 parts per million.
- Mercury (as Hg), not more than 1 part per million.
- Total color, not less than 87 percent.

FD&C Yellow No. 5 was first listed for food use in the United States in 1916. In 1994, 799,531.4 kg of FD&C Yellow No. 5 dye and 441,000.9 kg of FD&C Yellow No. 5 lake were certified for use in the United States.

The World Health Organization/Food and Agriculture Organization Joint Expert Committee for the Evaluation of Food Additives (WHO/FAO JECFA) has also evaluated the safety of FD&C Yellow No. 5 used as a coloring agent in food. An average daily intake (ADI) of 0-7.5 mg/kg bw per day was assigned by JECFA in 1964 based on the extensive human toxicological information available that indicated FD&C Yellow No. 5 did not possess carcinogenic potential (see Table 2 below).

Table 2. Regulatory Approvals/Consumption Limits[1]	
USA	GMP (21 CFR 74.705)
EEC	GMP (EC Journal No. L237/13; 1994)
JECFA	ADI of 0-7.5 mg/kg (8th report, 1964)

Based on the long history of use of FD&C Yellow No. 5 in food, the many hazard assessments performed by the United States FDA and WHO/FAO JECFA, and the current regulatory status of FD&C Yellow No. 5, there is no compelling evidence that this substance should be further tested for human health endpoints in the EPA Chemical "Right to Know" Program.

2.4 STRUCTURAL CLASSIFICATION

FD&C Yellow No. 5 is principally the trisodium salt of 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[4-sulfophenyl-azo]-1H-pyrazole-3-carboxylic acid (USFDA-21 CFR 74.705).

2.5 INDUSTRIAL PRODUCTION

In order to manufacture FD&C Yellow No. 5, 4-amino-benzenesulfonic acid is diazotized using hydrochloric acid and sodium nitrite. The diazo compound is then coupled with 4,5-dihydro-5-oxo-1-(4sulfophenyl)-1H-pyrazole-3-carboxylic acid or with the methyl ester, the ethyl ester, or a salt of this carboxylic acid. The resulting dye is purified and isolated as the sodium salt.

[1] IACM, 2003

2.6 PHARMACOKINETICS AND METABOLISM

FD&C Yellow No. 5 undergoes bacterial azo reduction in the gastrointestinal tract of rats, rabbits, and humans [Allan & Roxon, 1974; Chung *et al*., 1978; Dubin & Wright, 1975; Roxon *et al*., 1967a; Roxon *et al*., 1967b; and Watabe *et al*., 1980]. Following reductive cleavage of the azo linkage by intestinal bacteria, sulfanilic acid and aminopyrazolone are produced. The pyrazolone fragment is further degraded by intestinal bacteria to yield a second molecule of sulfanilic acid. In rats, relatively small amounts of these metabolites are excreted in the urine with the majority being detected in the feces [Honohan *et al*., 1977].

Groups of Sprague-Dawley female rats were given single oral doses of aqueous solutions (1%) containing 2 to 25 mg of ^{14}C-tartrazine labeled in the 1-p-sulphophenyl ring. Urine and feces were collected at 24-hour intervals. Bile was collected from bile duct cannulated animals and blood was collected regularly from the orbital sinus. After 72 hours, animals were sacrificed an tissues from the liver, spleen, kidneys, stomach, small intestine, caecum, large intestine, and peri-uterine fat sample were subjected for radioassay. Total 72-hour urinary excretion of tartrazine was only 4.0%. Biliary excretion was less than 0.1% while there was only trace amounts of radioactivity in internal organs after 72 hours. In terms of metabolites, 21% of the total radioactivity was detected in the urine as sulfanilic acid. Twenty-four hours after dosing, approximately equal amounts of urine radioactivity (43-44%) was accounted for by sulfanilic acid and aminopyrazolone. The urinary radioactivity corresponded to 20% and 1.6% of the administered dose of tartrazine being excreted as sulfanilic acid and aminopyrazolone, respectively. Only a trace amount of intact tartrazine was detected in the urine [Honohan *et al*., 1977].

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

The melting point of FD&C Yellow No. 5 was calculated to be 350 °C using modeling software [MPBPVPWIN EPI Suite, 2000]. Substances of similar structure and molecular weight decompose on heating to temperatures >300 °C.

3.1.2 Boiling Point

The boiling point of FD&C Yellow No. 5 was calculated to be 870 °C [MPBPVPWIN EPI Suite, 2000]. Technically, data for this endpoint are not required given that this material is a solid and would likely decompose upon heating to elevated temperatures.

3.1.3 Vapor Pressure

The calculated vapor pressure for FD&C Yellow No. 5 has been reported to be 7.43 X 10-22 mm Hg at 25°C [MPBPVPWIN EPI Suite, 2000]. Given the high molecular mass of FD&C Yellow No. 5 (556.34) and the estimated Henry's law constant for azo dyes of 10^{-15} atm-m^3/mol it is highly unlikely that FD&C Yellow No. 5 would exhibit any significant (less than 0.001 mm Hg) vapor pressure. This is predicted by the MPBPVPWIN model. Based on these data, the vapor pressure is less than 1 X 10^{-20} mm Hg.

3.1.4 Octanol/Water Partition Coefficients

Log K_{OW} value for FD&C Yellow No. 5 is –10.17 [KOWWIN EPI Suite, 2000]. The experimental log K_{OW} value would be difficult to obtain by OECD methods given the large difference between water solubility and anticipated solubility in octanol. Based on the observations that FD&C Yellow No. 5 is freely soluble in water (200,000 mg/L) and

essentially insoluble in a relatively polar solvent like ethanol (10 mg/L) [Marmion, 1991], it is anticipated that the log K_{OW} value for this substances would exceed -6.0.

3.1.5 Water Solubility

FD&C Yellow No. 5 has a reported water solubility of 38,000 mg/L at 2 °C, 200,000 mg/L at 25 °C, and 200,000 mg/L at 60 °C [Marmion, 1991]. The solubility of FD&C Yellow No. 5 in 100% glycerol is 180,000 mg/L at 25 °C while the solubility in ethanol is reported to be 10 mg/L at 60 °C [Marmion, 1991, robust summary not included]. The solubility of FD&C Yellow No. 5 in octanol is expected to be less than 1 mg/L.

3.1.6 New Testing Required

None.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

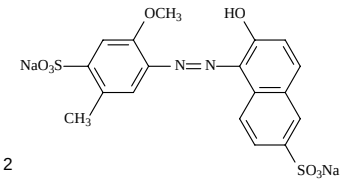
Direct and indirect photolysis experiments were conducted on the structurally related monoazo dye, FD&C Red No. 40[2], using two 15-watt low pressure lamps as the ultraviolet light source. Following 50 minutes of exposure to the lamps, FD&C Red No. 40 concentration decreased by 7% in the direct experiment. In the indirect experiment which used acetone as the sensitizer, the concentration of FD&C Red No. 40 decreased by 99% after 20 minutes [Pasin and Rickbaugh, 1991]. The calculated half-life for FD&C Yellow No. 5 in hydroxyl radical reactions is 3.5 hours [AOPWIN EPI Suite, 2000].

3.2.2 Stability In Water

FD&C Yellow No. 5 does not contain functional groups (*e.g.*, esters, amides, acetals, epoxides, lactones, *etc*.) that hydrolyze in water. The only potential reactivity in water would involve desulfonation of the aromatic sulfonic acid or its corresponding sulfonic acid salt. In aqueous acid (sulfuric acid), aromatic sulfonic acids desulfonate at temperatures of 100 to 175 ^{o}C. These conditions would not typically be encountered in the environment. Therefore, FD&C Yellow No. 5 and its corresponding salts are anticipated to be stable in water.

3.2.3 Biodegradation

The biodegradability of azo dyes ring-substituted with a carboxylic acid and two sulfonic acid groups consistently show that these substances are not absorbed onto activated sludge and, therefore, are not biodegradable [Shaul *et al.*, 1990]. Incubation of 1.0 or 5.0 mg/L of a structurally related azo dye, (1-naphthalenesulfonic acid, 4-hydroxy-3-[(4-



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sulfo-1-naphthalenyl)azo]-, disodium salt)[3] with activated sludge from a sewage treatment plant revealed that the concentration of dye remained essentially constant in the influent flow, primary effluent, and activated sludge effluent. Essentially no azo dye was absorbed by activated sludge. Two other azo dyes ring-substituted with sulfonic acid groups (Acid Orange No. 10 and Acid Red No. 1) exhibited a similar behavior in these experiments.

FD&C Yellow No. 5 was not predicted to be readily degradable by BIOWIN model calculations [AOPWIN EPI Suite, 2000].

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model Version 2.70 [ECOSAR EPI Suite, 2000]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log K_{OW}.

As expected, the model predicts that FD&C Yellow No. 5 is distributed completely to the water and soil compartments. Consistent with the extremely high water solubility and low log K_{OW} data, FD&C Yellow No. 5 showed no distribution to the fish compartment. These data are consistent with ecotoxicity data for aromatic sulfonic acid derivatives that demonstrate essentially no absorption and toxicity to fish even at concentrations exceeding 1000 mg/L.

3.2.5 New Testing Required

None.

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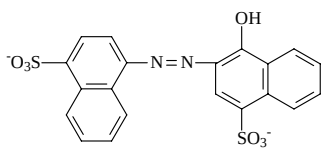
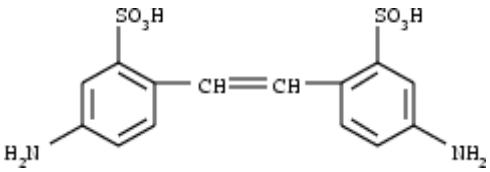
3 

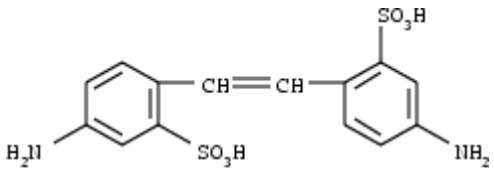
3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

Based on input parameters for molecular weight (556.34), water solubility (200,000 mg/L at 25 °C), the calculated 96-hour LC50 for FD&C Yellow No. 5 is 1.14 X 10^{14} mg/L [ECOSAR EPI Suite, 2000] indicates a very low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 5 is to a large extent, a function of the presence of aromatic sulfonic acid and carboxylic acid ring substituents. The extensive studies on the ecotoxicity of aromatic sulfonic acids indicate a very low order of toxicity to fish [Greim *et al.*, 1994]. Experimental LC50 values are available for stilbene sulfonic acids in which the N atom in the diazo dye is replaced by C. As indicated in Table 3 below, acute fish toxicity studies on salts of stilbene sulfonic acid derivatives result in a 96-hour LC50 value greater than 10,000 mg/L. Also, 48-hour and 72-hour LC50 concentrations of 200 and greater than 1000 mg/L, respectively have been reported [Greim *et al.*, 1994]. These values are consistent with calculated values.

Table 3

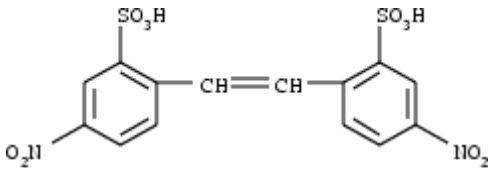
Name	Acute Toxicity to fish
2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid 	48-hour LC50: 200 mg/L
2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt	72-hour LC50: greater than 1000 mg/L



• 2 Na

2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, dipotassium salt

96-hour LC50: greater than 10,000 mg/L



• 2 K

Given the high-calculated LC50 values from the ECOSAR model, the experimentally measured toxicity of aromatic sulfonic acid derivatives, and the difficulties inherent in acute aquatic testing with dyes with very high extinction coefficients for a major portion of the visible-ultraviolet spectrum, no additional testing is warranted.

3.3.2 Acute Toxicity to Aquatic Invertebrates

The calculated 48-hour LC50 value for FD&C Yellow No. 5 in *daphnids* is 5.25 X 10^{13} mg/L based on input parameters for molecular weight (556.34), and water solubility (200,000 mg/L at 25 °C), [ECOSAR EPI Suite, 2000] indicating a low order of acute toxicity. The extensive water solubility and limited lipophilicity of FD&C Yellow No. 5 is to a large extent, a function of the presence of aromatic sulfonic acid ring substituents. The extensive studies on the ecotoxicity of aromatic sulfonic acids indicate a very low order of toxicity to aquatic invertebrates [Greim *et al.*, 1994]. An experimental 24-hour EC50 value with *Daphnia* for a stilbene sulfonic acid derivative, 2,2’-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, was greater than 100 mg/L [Greim *et al.*, 1994]. This value is consistent with calculated values.

3.3.3 Acute Toxicity to Aquatic Plants

Based on input parameters for molecular weight (556.34), and water solubility (200,000 mg/L at 25 °C), the calculated 96-hour EC50 for FD&C Yellow No. 5 with green algae is 1.63 X 10^{13} mg/L [ECOSAR EPI Suite, 2000] indicating a very low order of acute toxicity. In a 96-hour algal chronic toxicity test, a sulfonic acid substituted azo dye, stimulated population growth (26.4%) compared to control (algal assay medium) [Greene and Baughman, 1996]. In fact, of the 46 dyes tested, only one, an anthraquinone dye, produced and measurable toxicity in terms of decreased algal growth rates. Given the low-predicted acute toxicity of FD&C Yellow No. 5 to aquatic plants and the stimulation of plant growth resulting from the addition of a structurally related azo dye in an experimental acute toxicity test, it is not recommended that additional tests be performed.

3.3.4 New Testing Required

None.

3.4 HUMAN HEALTH TOXICITY

3.4.1 Acute Toxicity

In reports submitted to the World Health Organization, the acute oral LD50 in mice was reported to be 12,750 mg/kg bw [National Institute of Hygienic Sciences of Japan, 1964]. In rats, the LD50 by intraperitoneal injection was reported to be 2,000 mg/kg bw and the LD50 by intravenous injection was reported to be 1,000 mg/kg bw [Deutsche Forschungsgemeinschaft, 1957].

3.4.2 *In vitro* and *In vivo* Genotoxicity

3.4.2.1 In vitro

FD&C Yellow No. 5 tested negative in reverse mutation assay using TA1535, TA1537, TA1538, TA98, and TA100 with and without metabolic activation [Chung *et al*., 1981; Ishidate *et al*., 1984; Muzzall and Cook, 1979]. In one chromosomal aberration test, FD&C Yellow No. 5 tested positive at concentrations up to 2,500 micrograms/mL (approximately 5 mM) without metabolic activation [Ishidate *et al*., 1984].

In an *in vitro* UDS assay using rat hepatocytes, FD&C Yellow No. 5 tested negative at concentrations up to and including 2 X 10^{-6} M [Kornbrust and Barfknecht, 1985].

3.4.2.2 In vivo

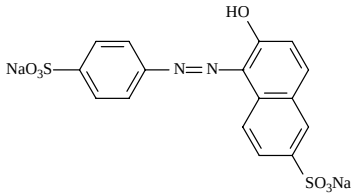
In an *in vivo* UDS assay, six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg/kg bw FD&C Yellow No. 5 *via* gavage. FD&C Yellow No. 5 did not induce unscheduled DNA synthesis at the dose level tested [Kornbrust and Barfknecht, 1985].

In a rodent micronucleus test, 10 ml/kg bw male rats were administered a single oral dose of 500 or 1000 mg/kg of the structurally related azo dye FD&C Yellow No. 6[4]. Bone marrow samples were taken at 24 and 48 hours later. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point in either species. [Westmoreland and Gatehouse, 1991].

3.4.3 Repeat Dose Toxicity

Groups of sixty male and sixty female mice each were administered 0, 0, 0.5, 1.5 or 5.0% FD&C Yellow No. 5 in the diet daily for 104 weeks. Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily, while detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for weeks 16-26 and monthly from week 26 until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (5.0%) and any animals with gross lesions or masses.

Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes, stomach, thymus, thyroid gland



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including parathyroid, trachea, and urinary bladder. Physical observations included hair loss, lacrimation, nasal discharge, staining of hair in the anogenital region and soft stools. None of these observations was attributed to administration of the test substance. Discolored urine and feces was reported at all treatment levels within one week of the study initiation. Mean body weights of both sexes were slightly lower than controls at the 5.0% treatment group for a number of sampling intervals, and male mice at the 1.5% treatment group were lower than controls for a number of sampling intervals. These differences were significantly lower in some intervals. Mean food consumption was significantly increased in male mice at the 5.0% treatment level. No statistically significant differences were reported for any of the hematological parameters. Common neoplastic, inflammatory, and degenerative lesions were reported amongst treated and control animals but were not considered to be treatment related. The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD&C Yellow No. 5. The no observable adverse effect level (NOAEL) of 5.0% providing an average daily intake of 8103 or 9753 mg/kg/day was established for male and female mice under the conditions of this study [Borzelleca and Hallagan, 1988b].

In a lifetime toxicity/carcinogenicity study, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD&C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents for 113 weeks. Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily with at least 5 hours between observations. Detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for the next 12 weeks and every 4 weeks thereafter until the end of the study. The intake of FD&C Yellow 5 was determined from body weight, food

consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on ten randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (2.0 or 5.0%) from each study and also on 10 rats randomly selected from each group for an interim sacrifice at 12 months. Histology was also performed on any animal with gross lesions or masses. Tissues examined included adrenal glands, aorta, blood smear, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, duodenum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes with epididymides, stomach, thymus, thyroid gland including parathyroid, trachea, urinary bladder, uterus.

During the *in utero* phase, there were no treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups. Slight decreases in body weight (4-5%) and slight increases in food consumption were noted in the F0 rats treated at dietary level of 5.0%. Two F0 female controls rats died during the *in utero* phase of the original study and one male and one female from the control and 5.0% group, respectively, died during the *in utero* phases of the high-dose study. There were no treatment related effects on pup survival.

In the F1 generation, a yellow tint was reported at all intake levels above 0.1%. At the 1.0% dietary level, group mean body weights at termination for both sexes were lower than the control animals, but the difference was only statistically significant for the females. In the high dose study (5.0% dietary level), group mean body weights were significantly lower in both sexes at termination. Food consumption was similar for control and treated animals at the 0.01, 1 or 2% dietary levels, but was slightly higher at the 5% level in the high-dose study, although not statistically significant. Hematological, clinical chemistry and urinalysis parameters did not differ significantly from the controls.

Necropsies at one year did not reveal any treatment-related gross or microscopic changes. At study termination, no treatment-related effects were reported on survival. No treatment-related changes were reported at gross necropsy. Histological evaluation revealed a variety of lesions, including neoplasms, present at similar incidences in control and treated animals. The authors considered the lesions to be spontaneous and not related to administration of the test material. The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD&C Yellow No. 5. A NOAEL of 5.0% providing an average daily intake of 2641 mg/kg/day and 3348 mg/kg/day for male and female rats, respectively, was reported under the conditions of this study [Borzelleca and Hallagan, 1988a].

3.4.4 Developmental Toxicity

In a guideline study performed by FDA, female Osborne-Mendel (FDA strain) rats (40-41 per group) were administered FD&C Yellow No. 5 *via* gavage at dose levels of 0, 60, 100, 200, 400, 600 or 1000 mg/kg bw/day for the first 19 days of gestation. On day 19, the animals were examined for gross abnormalities followed by euthanization. Caesarean sections were performed. The uterus was examined for presence and position of resorption sites and fetuses, number of corpora lutea and implantation sites. All live fetuses were promptly weighed, sexed, and examined. Crown-rump lengths were measured. Fetuses were divided and assigned to skeletal or soft tissue examination.

The authors reported no unusual behavior or external findings among the dosed females of any group. One female at the 60 mg/kg bw/day dose level died on gestation day 13 due to gavage difficulties. The mean daily food consumption of rats administered the 1000 mg/kg bw/day dose level was significantly greater than the controls. Initial body weight and maternal weight gain during gestation did not significantly differ between treated animals and controls. Pregnancy rate was similar among all groups.

No dose related findings were reported on fetal viability or fetal development. The incidence of sternebral variations was similar for all groups. The authors commented that the significant increase in food consumption observed in the highest dose group without a

corresponding effect on body weight indicated an effect on food utilization. The authors concluded that FD&C Yellow No. 5 was neither developmentally toxic nor teratogenic under the conditions of the study. The NOAEL for maternal and fetal toxicity was determined to be greater than 1000 mg/kg bw/day [Collins *et al*., 1990].

3.4.5 Reproductive Toxicity

In a lifetime toxicity/carcinogenicity study, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD&C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents for 113 weeks. During the *in utero* phase, there were no treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups [Borzelleca and Hallagan, 1988a].

3.4.6 New Testing Required

None.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
C.I. Acid Yellow 23 CAS No. 1934-21-0	Calc	Calc	Calc	Calc	A

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
C.I. Acid Yellow 23 CAS No. 1934-21-0	R, Calc	NA	R, Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
C.I. Acid Yellow 23 CAS No. 1934-21-0	R, Calc	R, Calc	R, Calc

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
C.I. Acid Yellow 23 CAS No. 1934-21-0	A	A	A, R	A	A	A

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties

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201-15133B

Robust Summaries for

C.I. Acid Yellow 23

CAS No. 1934-21-0

Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:
The International Association of Color Manufacturers/HPV Committee
1620 I Street, NW, Suite 925
Washington, DC 20006
Phone: 202-331-2325
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Robust Summaries

for C.I. Acid Yellow 23

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al*., 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 CHEMICAL AND PHYSICAL PROPERTIES

1.1 MELTING POINT

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for substance	FD&C Yellow 5
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Melting Point	350 °C
Decomposition	
Sublimation	

Remarks for Results	
Conclusion Remarks	
Remarks for General Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.2 BOILING POINT

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Boiling Point	870 °C
Pressure	
Pressure Unit	
Decomposition	
Remarks for Results	
Conclusion Remarks	
Remarks for General Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.3 VAPOR PRESSURE

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for substance	FD&C Yellow 5
Method/guideline	Calculated/Mean of Antoine & Grain
GLP	No
Year	
Remarks for Test Conditions	
Vapor Pressure	7.43 X 10-22 mm Hg
Temperature	25 °C
Decomposition	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVPWIN EPI Suite (2000) US Environmental Protection Agency.

1.4 N-OCTANOL/WATER PARTITION COEFFICIENTS

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for substance	FD&C Yellow No. 5
Method/guideline	Calculated
GLP	
Year	
Remarks for Test Conditions	
Log Pow	-10.17

Temperature	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) US Environmental Protection Agency.

1.5 WATER SOLUBILITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Purity not given
Method/guideline	Not given
GLP	Ambiguous
Year	1991
Remarks for Test Conditions	Not given
Value (mg/L) at temperature	38,000 mg/ml at 2 °C; 200,000 mg/ml at 25 °C; 200,000 mg/ml at 60 °C
Description of Solubility	Not given
pH value and concentration at temp	
pKa value at 25 Celsius	
Remarks for Results	
Conclusion Remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Marmion D.M. (1991) Handbook of U.S. Colorants: Foods, Drugs, and Cosmetics and Medical Devices. 3rd Ed. New York, John Wiley & Sons, Inc.

2 ENVIRONMENTAL FATE AND PATHWAYS

2.1 PHOTODEGRADATION

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Data are for structurally related substance 2-Naphthalenesulfonic acid, 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-, disodium salt (FD&C Red 40)
Method/guideline	Not given
Test Type	Experimental
GLP	Ambiguous
Year	1991
Light Source	15-watt General Electric germicidal lamps
Light Spectrum (nm)	Ultraviolet
Relative Intensity	
Spectrum of Substance	
Remarks for Test Conditions	The concentration of the dye solution was measured before and after the photolysis using the Hewlett-Packard 8452A diode-array UV/Visible Spectrophotometer. Red 40 was prepared in an initial concentration of 5 mg/l. In the first part of the study, photolysis experiments were conducted using two 15-W (30 Watts total) General Electric germicidal lamps as the ultraviolet light source. The distance between the light source and the reaction vessels was approximately 2.5 cm. Both direct photolysis and indirect photolysis experiments were conducted. The indirect photolysis experiment used acetone as the sensitizer for indirect photodegradation.
Concentration of Substance	5 mg/L
Temperature	
Direct photolysis	7% degradation after 50 minutes
Halflife t1/2	
Degradation % after	
Quantum yield	
Indirect photolysis	99% degradation after 20 minutes
Sensitizer	acetone

Concentration of sensitizer	5 mg/L
Rate constant	
Degradation %after	
Breakdown products	
Remarks field for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Pasin B. and Rickabaugh J. (1991) Destruction of Azo Dyes by Sensitized Photolysis. Hazard. Ind. Wastes, 359-367.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5
Method/guideline	Calculation
Test Type	AOPWIN
GLP	
Year	
Light Source	
Light Spectrum (nm)	
Relative Intensity	
Spectrum of Substance	
Remarks for Test Conditions	
Concentration of Substance	
Temperature	
Direct photolysis	
Halflife t1/2	3.5 hours
Degradation % after	
Quantum yield	
Indirect photolysis	

Sensitizer	
Concentration of sensitizer	
Rate constant	
Degradation %after	
Breakdown products	
Remarks field for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) US Environmental Protection Agency.

2.2 BIODEGRADATION

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Data are for structurally related sulfonic acid C.I. Acid Red No. 14.
Method	Not given
Test Type	
GLP	Ambiguous
Year	1993
Contact time (units)	24 hour
Innoculum	Activated sludge
Remarks for Test Conditions	Screened raw wastewater was used as the influent in three pilot scale activated sludge biological treatment systems. Each water soluble dye was tested at doses of 1 mg/L for low spike systems and 5 mg/L for high spike systems of influent flow. Before the data collection, dye analytical recovery studies were conducted by dosing the purified dye compound into organic free water, influent wastewater, and mixed liquor. These studies were run in duplicate and each recovery study was

	repeated at least once to ensure that the dye compound could be extracted. Purified dye standards were analytically prepared from the commercial dye product by repeated recrystallization. The INF, primary effluent (PE), and ASE were filtered and the filtrate was passed through a column packed with resin. The filter paper and resin were soaked in an ammonia acetonitrile solution and then Soxhlet extracted with ammonia-acetonitrile. The extract was concentrated and brought up to 50 mL volume with a methanol/dimethylformamide solution. The mixed liquor samples were separated into two components, the filtrate or soluble fraction (SOL) and the residue (RES) fraction. The SOL fraction was processed similar to these samples but he resin adsorption step was omitted. All extracted samples were analyzed by HPLC with and ultraviolet-visible detector. Total suspended solids analyses were also performed on the INF, PE, ML, and ASE samples. All systems were operated for at least three times the solids retention time to ensure acclimation prior to initiation of data collection. All samples were 24 hour composites made up of 6 grab samples collected every 4 hour and stored at 4 °C.
Results	Percent recovery as measured: Organic Free Water: 101% at 1 mg/L and 90% at 5 mg/L; Wastewater: 98% at 1mg/L and 97% at 5 mg/L; Mixed Liquor: 88% at 1mg/L and 92% at 5 mg/L Mass Balance Data Summary: Low spike: 116% recovered, 1% adsorbed; High spike: 148% recovered, less than 1% adsorbed.
Classification	
Remarks fields for results	Since the majority of the test substance was recovered, the authors assumed that this compound was not biodegraded. The authors based this assumption on preliminary data indicating little or no problems in recovering the compounds from the sample matrix. Additionally the results also indicate that the material was not adsorbed. The authors attributed the high sulfonic acid substitution on the test substance as the reason why the material was not removed by the microbial cells or cell byproducts and subject to aerobic biodegradation.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Shaul G.M., Holdsworth T.J., Dempsey C.R., and Dostal K.A. (1990) Fate of water soluble azo dyes in the activated sludge process. Chemosphere 22, p107-119.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5

Method	
Test Type	Calculated
GLP	
Year	
Contact time (units)	
Innoculum	
Remarks for Test Conditions	
Results	
Classification	Not readily biodegradable
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	BIOWIN EPI Suite (2000) US Environmental Protection Agency.

2.3 FUGACITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP

Year	
Remarks for Test Conditions	
Media	Air
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration Remarks	3.05E-13%
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Water
absorption coefficient	
Desorption	
Volatility	
Model data and results	

Estimated Distribution and Media Concentration Remarks	51.8%
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Soil
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration Remarks	48.1%
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5
Model Conditions	25 C, 100,000 lbs.
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used (title, version, date)	EQC V 2.70 Level III
Input parameters	MW, log Kow, water solubility, MP & VP
Year	
Remarks for Test Conditions	
Media	Sediment
absorption coefficient	
Desorption	
Volatility	
Model data and results	
Estimated Distribution and Media Concentration	0.0981%
Remarks	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) US Environmental Protection Agency. Level III. Fugacity.

3 ECOTOXICITY

3.1 ACUTE TOXICITY TO FISH

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Data are for sulfonic acid derivative 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid
Method/guideline	
Test Type	Experimental
GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	48 hour
Remarks for Test Conditions	
Observations on precipitation	
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	LC50 = 200 mg/L
Reference substances (if used)	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185.

	Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Data are for sulfonic acid derivative 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt
Method/guideline	
Test Type	Experimental
GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	72 hour
Remarks for Test Conditions	
Observations on precipitation **Nominal concentrations as mg/L** **Measured concentrations as mg/L** **Unit**	
Endpoint value	LC50 greater than 1000 mg/L
Reference substances (if used) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Data are for sulfonic acid derivative 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, dipotassium salt
Method/guideline	
Test Type	Experimental
GLP	Ambiguous
Year	Not given
Species/Strain/Supplier	Fish
Analytical monitoring	
Exposure period (unit)	96 hour
Remarks for Test Conditions	
Observations on precipitation **Nominal concentrations as mg/L** **Measured concentrations as mg/L** **Unit**	
Endpoint value	LC50 greater than 10,000 mg/L
Reference substances (if used) **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	
Analytical monitoring	
Exposure period (unit)	96 hour
Remarks for Test Conditions	Input parameters: Molecular weight, Water solubility, 200,000 mg/L at 25 °C
Observations on precipitation	
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	LC50 = 1.14 E+14 mg/L
Reference substances (if used)	
Remarks fields for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Data are for sulfonic acid derivative 2,2'-(1,2-ethene-diyl)bis(5-amino)-benzenesulfonic acid, disodium salt
Method/guideline	
Test Type	Experimental
GLP	
Year	
Analytical procedures	
Species/Strain	*Daphnia magna*
Test details	24 hour
Remarks for Test Conditions	
Nominal concentrations as mg/L **Measured concentrations as mg/L** **Unit**	
EC50, EL50, LC0, at 24,48 hours **Biological observations**	EC50 = 100 mg/L
Control response satisfactory? **Appropriate statistical evaluations?** **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Alstoffe, (1992) Daten zur Beurteilung der Wirkung auf Mensch and Umwelt-Satensatze, Verband der Chemischen Industrie, Frankfurt 1992. Schön N. (1991) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 3(3), 183-185. Schön N. (1992) Altsoff-Grunnddatensätze-Liste der bisher publizierten Grunnddatensätze UWSF-Z. Umwelchem. Ökotox, 4(6), 343-345.

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Analytical procedures	
Species/Strain	*Daphnia magna*
Test details	48 hours
Remarks for Test Conditions	Input parameters: Water solubility, 200,000 mg/L at 25 °C; Molecular weight 556.34
Nominal concentrations as mg/L **Measured concentrations as mg/L** **Unit**	
EC50, EL50, LC0, at 24,48 hours **Biological observations**	EC50 = 5.25 E+13 mg/L
Control response satisfactory? **Appropriate statistical evaluations?** **Remarks fields for results**	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

3.3 ACUTE TOXICITY TO AQUATIC PLANTS

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	The test substance was an unidentified sulfonic acid substituted azo dye.
Method/guideline	
Test Type	Experimental
GLP	Ambiguous
Year	1996
Species/Strain/Supplier	Green algae, *Selenatrum capricornutum*
Endpoint basis	
Exposure period (duration)	96 hour
Analytical monitoring	
Remarks for Test Conditions	Algal chronic toxicity test were performed according the method of EPA, 1988. Three replicates were performed for each dye at a nominal concentration of 1 mg/l for the active colorant. One ml of dye stock solution was added to 50 mg/l of algal assay medium in 125 ml Erlenmeyer flasks. *S. capricornutum* in continuous culture provided the initial innoculum (10,000 algal cells/ml). The cells were incubated in the solution for 96 hours. The diluent and negative control were algal assay medium. AAM was prepared by adding 1 ml from each of five stock solutions to 900 ml of deionized water. After spiking, the total volume was brought to 1 liter with deionized water. Population growth was used to establish potential toxicity. If the dye inhibited algal growth by more than 50% of that of the negative controls, a definitive test using several dilutions of the dye was performed to allow for determination of an EC50 concentration.
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	Average yield: 36.6% with 95% C.I. (34.9-38.4).
NOEC, LOEC or NOEL, LOEL	
Biological observations	26.4% stimulation of population growth compared to control.
Control response satisfactory?	Yes
Appropriate statistical evaluations?	Yes, Dunnett's test
Remarks fields for results	Not statistically significant.

Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Greene J. C. and Baughman G.L. (1996) Effects of 46 dyes on population-growth of fresh-water green-alga *selenastrum-capricornutum*. Textile Chemist And Colorist, 28, 23-30. Green J.D. et al. (1988) Protocols for short term toxicity screening of hazardous waste sites. Report to EPA 600/3-88-029. U.S. Environmental Protection Agency. Corvallis, Oregon.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5
Method/guideline	ECOSAR
Test Type	Calculated
GLP	
Year	
Species/Strain/Supplier	Green algae
Endpoint basis	
Exposure period (duration)	96 hour
Analytical monitoring	
Remarks for Test Conditions	Input parameters: Water solubility - 200,000 mg/L at 25 °C; Molecular weight 556.34
Nominal concentrations as mg/L	
Measured concentrations as mg/L	
Unit	
Endpoint value	EC50 = 1.63 E+13 mg/L
NOEC, LOEC or NOEL, LOEL	
Biological observations	
Control response satisfactory?	
Appropriate statistical evaluations?	
Remarks fields for results	

Conclusion remarks

Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	ECOSAR EPI Suite (2000) US Environmental Protection Agency (Nabholz V. and G. Cash, 1998).

4 HUMAN HEALTH TOXICITY

4.1 ACUTE TOXICITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Not given
Method/guideline	Not given
Test Type	Acute Toxicity LD50
GLP	No
Year	1957
Species/Strain	Rat
Sex	Not reported
# of animals per sex per dose	Not given
Vehicle	Not given
Route of administration	Intraperitoneal
Remarks for test conditions	
Value LD50 or LC50 with confidence limits	2,000 mg/kg bw
Number of deaths at each dose level	
Remarks for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Deutsche Forschungsgemeinschaft, Bad Godesberg, Federal Republic of Germany, Farbstoff Kommission (1957) Mitteilung 6.

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Not given
Method/guideline	Not given

Test Type	Acute Toxicity LD50
GLP	No
Year	1957
Species/Strain	Rat
Sex	Not reported
# of animals per sex per dose	Not given
Vehicle	Not given
Route of administration	Intravenous
Remarks for test conditions	
Value LD50 or LC50 with confidence limits	1,000 mg/kg bw
Number of deaths at each dose level	
Remarks for results	
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Deutsche Forschungsgemeinschaft, Bad Godesberg, Federal Republic of Germany, Farbstoff Kommission (1957) Mitteilung 6.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Not given
Method/guideline	Not given
Test Type	Acute Toxicity LD50
GLP	No
Year	1964
Species/Strain	Mice
Sex	Not reported
# of animals per sex per dose	Not given
Vehicle	1% gum arabic

Route of administration	Oral
Remarks for test conditions	
Value LD50 or LC50 with confidence limits **Number of deaths at each dose level** **Remarks for results**	12,750 mg/kg bw
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	National Institute of Hygienic Sciences of Japan. Unpublished data submitted to WHO, 1964 cited in ILSI report on FD&C Yellow 5 6/2/83.

4.2 GENETIC TOXICITY

4.2.1 In vitro Genotoxicity

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5; Purity not given
Method/guideline	Ames plate incorporation and liquid pre-incubation
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1981
Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA1538, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/concentration levels	0.005- 5.0 mg/plate

Statistical Methods	Not given
Remarks for test conditions	Reverse mutation tests were carried out using *S. typhimurium* strains TA1535, TA 1537, TA1538, TA98, TA100. Plate incorporation tests were conducted according to Ames *et al*., with the Andrews et al. modifications. Duplicates were performed at each of the six concentrations of the test substance. Mutagenic compounds were assayed using duplicate plates. A substance was considered positive when the number of revertants above background was at least twice the value of the historical control mean or twice the value of the current control mean, whichever was greater and a dose response curve could be generated. Positive controls without metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA97 and TA1535), and 4-nitro-o-phenylenediamine (TA98). The positive controls were sodium azide, 9-aminoacridine, 2-nitrofluorene, and 2-aminoanthracene.
Result	Negative
Cytotoxic concentration	5.0 mg/plate for plate-incorporation, and .5 mg/ml for pre-incubation test.
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	The test substance was negative in the AMES assa;y for reverse mutation using *Salmonella typhimurium* TA1535, TA 1537, TA1538, TA98, TA100.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Chung K.T., Fulk G.E., & Andrews A.W. (1981) Mutagenicity testing of some commonly used dyes. Applied and Environmental Microbiology 42, 641-648.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5; Purity not given
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	No
Year	1979

Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA98, TA100
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/concentration levels	10-250 mg/plate
Statistical Methods	Not given
Remarks for test conditions	The test substance was dissolved in DMSO. The test was considered positive if 2 fold increase in revertants was observed. Positive controls included 9-aminoacridine; 2-aminoflourine; N-methyl-N-nitrosoguanidine.
Result	Negative
Cytotoxic concentration	Not given
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	No evidence of genotoxicity was reported.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Muzzall J.M. and Cook W.I. (1979) Mutagenicity test of dyes used in cosmetics with the Salmonella/mammalian microsome test. Mutations Research 67, 1-8.a.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5; Purity not given
Method/guideline	Ames
Test Type	Reverse mutation
System of Testing	Bacterial
GLP	Ambiguous
Year	1984
Species/Strain	*Salmonella typhimurium* TA1535, TA 1537, TA98, TA100, TA92, TA94
Metabolic Activation	Rat liver microsome fraction S9 from Aroclor induced rats
Doses/concentration levels	Up to 5.0 mg/ml
Statistical Methods	Not given
Remarks for test conditions	Reverse mutation tests were carried out using *S. typhimurium* strains TA92, TA1535, TA100, TA1537, TA94 and TA98. Cells

	cultured overnight were pre-incubated with the test substance and the S-9 mix for twenty minutes at 37 degrees Celsius prior to plating. Duplicates were performed at each of the six concentrations of the test substance. The number of revertant colonies were counted following incubation for two days. Negative controls were either untreated plates or solvent. Positive results were determined if the number of colonies found was twice the number in the control. If the test was positive and a dose response relationship was not detected, additional experiments at different doses or induced mutation frequency assays were performed.
Result	Negative
Cytotoxic concentration	5.0 mg/ml was the highest non-cytotoxic dose used in the experiment.
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	The test substance was negative in the AMES assay for reverse mutation using *Salmonella typhimurium* TA1535, TA 1537, TA98, TA100, TA92, TA94.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Ishidate, M., Sofuni, T., Yoshikawa, K., Hauashi, M., Nohmi, T., Sawada, M. and Matsuoka. (1984). Primary Mutagenicity Screening of Food Additives Currently Used in Japan. Fd. Chem. Toxic. 22(8) 623-636.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5; Purity not given
Method/guideline	Chromosomal aberration test was carried out using a Chinese hamster fibroblast cell line, CHL. The cells were exposed to 3 different doses for 24 and 48 hours. No metabolic activation system was applied.
Test Type	Chromosomal aberration test
System of Testing	Chinese hamster fibroblast cell line CHL.
GLP	Ambiguous
Year	1984
Species/Strain	Chinese hamster fibroblast cell line CHL.
Metabolic Activation	None
Doses/concentration levels	up to 2.5 mg/ml

Statistical Methods	Not available
Remarks for test conditions	Chromosomal aberration tests were carried out using the Chinese hamster fibroblast line. Cells were exposed to the test substance at three different doses for 24 and 48 hour. No metabolic activation was employed. The maximum dose used for each test substance was found in a preliminary test to determine the dose required for 50% cell-growth inhibition. Colcemid at a final concentration of 0.2 ug/ml was added to the culture two hours prior to cell harvesting. The cells were prepared for viewing on slides. One hundred visible metaphases were observed under the microscope and the incidence of polyploid cells and structural chromosomal aberrations (including chromosome and chromatid gaps, breaks, exchanges, ring formations, fragmentations and others) were recorded. Negative controls included untreated cells and solvent treated cells. The incidence of aberrations in the negative controls was generally less than 3.0%. The results were considered negative if less than 4.9%, equivocal if between 5.0-9.9%, and positive if more than 10%. If dose response relationships were not observed, additional experiments were carried out at similar dose levels. The maximum dose for positive results represents the dose at which the maximum effect was obtained. For quantitative evaluation of the clastogenic potential, the D20 was calculated, which is the dose (mg/ml) at which structural aberrations (including gaps) were detected in 20% of the metaphases observed. In addition, the TR value was calculated, which indicates the frequency of cells with exchange-type aberrations per unit dose (mg/ml). These values are relatively high for chemicals that show carcinogenic potential in animals.
Result	The test substance was shown to be positive (23% total incidence of cells with aberrations) in chromosomal aberration test at 48 hours. TR value was 3.5 and D20 = 1.8. Weakly positive at 24 hour (11.0%, total incidence of cells with aberrations) The results were considered positive if the total incidence of cells with aberrations (including gaps) was 10.0% or more. Two percent (2%) reported as polyploid.
Cytotoxic concentration	Not given
Genotoxic effects	Positive
Appropriate statistical evaluations?	None given
Remarks for results	Positive
Conclusion remarks	C.I. Acid Yellow 23 tested positive in the chromosomal aberration test using Chinese hamster fibroblasts.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.

References	Ishidate, M., Sofuni, T., Yoshikawa, K., Hauashi, M., Nohmi, T., Sawada, M. and Matsuoka. (1984) Primary Mutagenicity Screening of Food Additives Currently Used in Japan. Fd. Chem. Toxic. 22(8) 623-636.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5; 94% purity
Method/guideline	Wiliams, 1977
Test Type	Unscheduled DNA Synthesis
System of Testing	Rat hepatocytes
GLP	Ambiguous
Year	1985
Species/Strain	Rat/Sprague-Dawley
Metabolic Activation	None
Doses/concentration levels	2 X 10-3 2 X 10-4 2 X 10-5 2 X 10-6
Statistical Methods	None given
Remarks for test conditions	Hepatocytes from rats were isolated and cultured according to the two step in situ liver perfusion model (Malansky and Williams, 1982). Viable hepatocytes (2 X 10+5) were seeded in wells and incubated for 4 hours with [H3]-thymidine (10 uCi/ml) and the test substance (prepared in either DMSO or water) according to a procedure similar to Williams, 1977. Control incubations were conducted with and without DMSO. The authors state that DMSO had no effect on DNA repair. Two experiments were conducted. DNA repair was quantified by the autoradiographic determination of incorporated [3H]-thymidine. Net nuclear grains (NNG) were determined by counting the number of grains in each nuclei and subtracting the average number of grains present in the three equal size adjacent cytoplasmic areas. Average NNG counts of 5 or more were assumed to constitute a positive response, because these differed from the control response by greater than 2 standard deviations. In the negative controls, NNG counts ranged from -0.6- to -2.8 and from -0.9 to -2.1 for no solvent and 1% DMSO incubations, respectively. The proportion of cells with greater than or equal to 5 NNG was less than or equal to 8.1% for all control incubations. Therefore NNG below zero were considered negative responses. Concentrations of dyes producing 90% or greater detachment of the hepatocytes from the coverslips

	were assumed to be toxic and not counted.
Result	The positive control was Solvent Yellow 3 (*o*-aminoazotoluene). Experiment 1 Conc Avg NNG % >5NNG 2 X 10-3 -1.7 (+/-2.6) 5 2 X 10-4 -2.4 (+/-3.3) 5 2 X 10-5 -2.4 (+/-3.2) 2 2 X 10-6 -2.0 (+/-2.8) 3 Experiment 2 Conc Avg NNG % greater than 5NNG 2 X 10-3 -2.2 (+/-
Cytotoxic concentration	Greater than 2 X 10-3
Genotoxic effects	Negative
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	C.I. Acid Yellow 23 did not induce unscheduled DNA synthesis in an *in vitro* assay using rat hepatocytes isolated from the livers of Sprague-Dawley rats.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Kornbrust D. and Barfknecht T. (1985) Testing Dyes in HPC/DR systems. Enviromental Mutagenesis 7, 101-120.

4.2.2 In vivo Genotoxicity

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	Data are for structurally related substance FD&C Yellow No. 6
Method/guideline	Rodent Micronucleus Test
Test Type	Rodent Micronucleus
GLP	Ambiguous

Year	1991
Species/Strain	Rat/PVG
Sex	Male
Route of administration	Oral-Gavage
Doses/concentration levels	10 ml/kg bw
Exposure period	Single dose
Remarks for test conditions	Male PVG rats received a single oral dose of 500, or 1000 mg/kg of the test substance. Bone marrow samples were taken at 24 and 48 hours later.
Effect on mitotic index or PCE/NCE ratio by dose level and sex	
Genotoxic effects	No significant increase in the frequency of micronucleated polychromatic erythrocytes at either time point and in either species was reported. Additionally, there was reported increase in the % PE (polychromatic erthyrocytes).
NOEL (C)/ LOEL (C)	
Appropriate statistical evaluations?	Yes.
Remarks for results	No effects.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report which meets basic scientific principles.
References	Westmoreland C. and Gatehouse D.G. (1991) The differential clastogenicity of Solvent Yellow 14 and FD & C Yellow No. 6 in vivo in the rodent micronucleus test (observations on species and tissue specificity). Carcinogenesis 12 (8), 1403-8.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	94% purity
Method/guideline	Mirsalis and Butterworth, 1980
Test Type	Unscheduled DNA Synthesis
GLP	Ambiguous
Year	1985
Species/Strain	Rat/Sprague Dawley
Sex	Male

Route of administration Oral-Gavage

Doses/concentration levels 500 mg/kg bw

Exposure period 2 hr; 15 hr

Remarks for test conditions Six to eight male Sprague-Dawley rats weighing 200-300 g were administered 500 mg acid yellow 23/kg bw *via* gavage. The control animal was administered corn oil only. Animals were killed at two time points, 2 hours and 15 hours. If negative results were obtained at time point 1 and time point 2, the *in vivo* testing was terminated and considered to be negative. If the initial test at time point 1 yielded a positive response, the test substance was retested at that time point. If another positive response was observed, the test was considered positive. Time points are the time the test substance was administered prior to the start of liver perfusion and isolation of hepatocytes.

Hepatocytes from rats were isolated and cultured according to the two step *in sit*u liver perfusion model (Malansky and Williams, 1982). Viable hepatocytes (2 X 10+5) were seeded in wells and incubated for 4 hours with [H3]-thymidine (10 uCi/ml) and the test substance (prepared in either DMSO or water) according to a procedure similar to Williams, 1977. Control incubations were conducted with and without DMSO. The authors state that DMSO had no effect on DNA repair.

DNA repair was quantified by the autoradiographic determination of incorporated [3H]-thymidine. Net nuclear grains (NNG) were determined by counting the number of grains in each nuclei and subtracting the average number of grains present in the three equal size adjacent cytoplasmic areas. Average NNG counts of 5 or more were assumed to constitute a positive response, because these differed from the control response by greater than 2 standard deviations. In the negative controls, NNG counts ranged from -0.6- to -2.8 and from -0.9 to -2.1 for no solvent and 1% DMSO incubations, respectively. The proportion of cells with greater than or equal to 5 NNG was less than or equal to 8.1% for all control incubations. Therefore NNG below zero were considered negative responses. Concentrations of dyes producing 90% or greater detachment of the hepatocytes from the coverslips were assumed to be toxic and not counted.

The positive control was Solvent Yellow 3 (o-aminoazotoluene).

Effect on mitotic index or PCE/NCE ratio by dose level and sex

Experiment 1

Dose (mg/kg bw)	Time	Avg NNG	% >5NNG
500	2 hr	-2.6 (+/-3.7)	2
	15 hr	-1.3 (+/-2.6)	2

Genotoxic effects Negative

NOEL (C)/ LOEL (C)	Greater than 500 mg/kg bw
Appropriate statistical evaluations?	None given
Remarks for results	Negative
Conclusion remarks	C.I. Acid Yellow 23 did not induce unscheduled DNA synthesis in an invivo assay using rat hepatocytes isolated from the livers of Sprague Dawley rats.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Kornbrust D. and Barfknecht T. (1985) Testing Dyes in HPC/DR systems. Enviromental Mutagenesis 7, 101-120.

4.3 REPEATED DOSE TOXICITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5; 90% purity; 10% intermediates or volatile matter
Method/guideline	Chronic Toxicity/Carcinogenicity Study
GLP	Yes
Year	1988
Species/Strain	Rat/Charles River CD
Sex	Male and Female
Route of administration	Oral-Diet
Doses/concentration levels	0, 0.1, 1.0, or 2.0% (original study) 0, 5.0% (high dose study)
Exposure period	113 weeks (males) or 114 weeks (females) (original study); 122 weeks (males) or 125 weeks (females) high-dose study
Frequency of treatment	Daily
Control Group	Yes, 2 concurrent controls (original study); 1 concurrent control (high-dose study)
Post exposure observation period	
Remarks for test conditions	In the *in utero* phase, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD & C Yellow No. 5 in the

diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents.

Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily with at least 5 hours between observations. Detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for the next 12 weeks and every 4 weeks thereafter until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on ten randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (2.0 or 5.0%) from each study and also on 10 rats randomly selected from each group for an interim sacrifice at 12 months. Histology was also performed on any animal with gross lesions or masses.

Tissues examined included adrenal glands, aorta, blood smear, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, duodenum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes with epididymides, stomach, thymus, thyroid gland including parathyroid, trachea, urinary bladder, uterus.

NOAEL(NOEL) 5.0 % (Males: 2641 mg/kg/d and Females: 3348 mg/kg/day)

LOAEL(LOEL) Not determined

Actual dose received by dose level and sex Males: 48, 491, 984 or 2641 mg/kg/day; Females: 58, 589, 1225 or 3348 mg/kg/day

Toxic response/effects by dose level *In utero*

There were no compound-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups. Slight decreases in body weight (4-5%) and slight increases in food consumption were noted in the F0 rats treated at dietary level of 5.0%. Two F0 female controls rats died during the *in utero* phase of the original study and one male and one female from the control and 5.0% group, respectively, died during the *in utero* phases of the high-dose study. There were no compound-related effects on pup

	survival. In the F1 generation, a yellow tint was reported at all intake levels above 0.1%. At the 1.0% dietary level, group mean body weights at termination for both sexes were lower than the control animals, but the difference was only statistically significant for the females. In the high dose study (5.0% dietary level), group mean body weights were significantly lower in both sexes at termination. Food consumption was similar for control and treated animals at the 0.01, 1 or 2% dietary levels, but was slightly higher at the 5% level in the high-dose study, although not statistically significant. Hematological, clinical chemistry and urinalysis parameters did not differ significantly from the controls. Necropsies at one year did not reveal any treatment-related gross or microscopic changes. At study termination, no treatment-related effects were reported on survival. No treatment-related changes were reported at gross necropsy. Histological evaluation revealed a variety of lesions, including neoplasms, present at similar incidences in control and treated animals. The authors considered the lesions to be spontaneous and not related to administration of the test material.
Appropriate statistical evaluations?	Yes, F-test, Anova
Remarks for results	The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD & C Yellow No. 5.
Conclusion remarks	The NOAEL of 5.0% providing an average daily intake of 2641 mg/kg/d and 3348 mg/kg/d for male and female rats, respectively, under the conditions of this study.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Borzelleca J. and Hallagan J. (1988a) A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (Tartazine) in rats. Fd Chem Toxic 26, 179-187.
CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5; 90% purity; 10% intermediates or volatile matter
Method/guideline	Chronic Toxicity/Carcinogenicity Study
GLP	Yes
Year	1988
Species/Strain	Mice/Charles River CD-1
Sex	Male and Female

Route of administration	Oral-Diet
Doses/concentration levels	0, 0.5, 1.5, or 5.0%
Exposure period	104 weeks
Frequency of treatment	Daily
Control Group	Yes
Post exposure observation period	
Remarks for test conditions	Groups of sixty male and sixty female mice each were administered 0, 0, 0.5, 1.5 or 5.0% FD & C Yellow No. 5 in the diet daily for 104 weeks. Animals were housed individually and fed the test diet *ad libitum*. Clinical observations were recorded twice daily, detailed physical examinations and palpations for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for weeks 16-26 and monthly from week 26 until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (5.0%) and any animals with gross lesions or masses. Tissues examined included adrenal glands, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes, stomach, thymus, thyroid gland including parathyroid, trachea, and urinary bladder.
NOAEL(NOEL)	5.0 % (8103 mg/kg/day)
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	M: 714, 2173 or 8103; F: 870, 2662 or 9735 mg/kg/day
Toxic response/effects by dose level	Physical observations included hair loss, lacrimation, nasal discharge, staining of hair in the anogenital region and soft stools. None of these observations was attributed to administration of the test substance. Discolored urine and feces was reported at all treatment levels within one week of the study initiation. Mean body weights of both sexes were slightly lower than controls at the 5.0% treatment group for a number of sampling intervals, and male mice at the 1.5% treatment group were lower than controls for a number of sampling intervals. These differences were significantly lower in

	some intervals. Mean food consumption was significantly increased in male mice at the 5.0% treatment level. No statistically significant differences were reported for any of the hematological parameters. Common neoplastic, inflammatory, and degenerative lesions were reported amongst treated and control animals but were not considered to be treatment related.
Appropriate statistical evaluations?	Yes, F-test, Anova
Remarks for results	The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD & C Yellow No. 5.
Conclusion remarks	The NOAEL of 5.0% providing an average daily intake of 8103 or 9753 mg/kg/d was established for male and female mice under the conditions of this study.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Borzelleca J. and Hallagan J. (1988b) A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (Tartazine) in mice. Fd Chem Toxic 26, 189-194.

4.4 DEVELOPMENTAL TOXICITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow No. 5; 92.7% purity
Method/guideline	FDA Teratology Study
Test Type	
GLP	Yes
Year	1990
Species/Strain	Rat/Osborne-Mendel (FDA strain)
Sex	Female
Route of administration	Oral-Gavage

Duration of test	19 days
Doses/concentration levels	0, 60, 100, 200, 400, 600 or 1000 mg/kg bw/day
Exposure period	19 days
Frequency of treatment	Daily
Control Group and treatment	Yes
Remarks for test conditions	Female Osborne-Mendel (FDA strain) rats (40-41 per group) were administered FD & C Yellow No. 5 via gavage at dose levels of 0, 60, 100, 200, 400, 600 or 1000 mg/kg bw/day for the first 19 days of gestation. On day 19, the animals were examined for gross abnormalities followed by euthanization. Caesarean sections were performed. The uterus was examined for presence and position of resorption sites and fetuses, number of corpora lutea and implantation sites. All live fetuses were promptly weighed, sexed, and examined. Crown-rump lengths were measured. Fetuses were divided and assigned to skeletal or soft tissue examination.
NOAEL(NOEL) maternal toxicity	Greater than 1000 mg/kg bw/day
LOAEL(LOEL) maternal toxicity	Not determined
NOAEL (NOEL) developmental toxicity	Greater than 1000 mg/kg bw/day
LOAEL (LOEL) developmental toxicity	Not determined
Actual dose received by dose level and sex	0, 60, 100, 200, 400, 600 or 1000 mg/kg bw/day
Maternal data with dose level	No unusual behavior or external findings were reported. One female at the 60 mg/kg bw/day dose level died on gestation day 13 due to gavage difficulties. The mean daily food consumption of rats administered the 1000 mg/kg bw/day dose level was significantly greater than the controls. Initial body weight and maternal weight gain during gestation did not significantly differ between treated animals and controls. Pregnancy rate was similar among all groups.
Fetal data with dose level	No dose related findings were reported on fetal viability or fetal development. The incidence of sternebral variations was similar for all groups.
Appropriate statistical evaluations?	Yes, ANOVA, Fisher's Exact Test, t-test.
Remarks for results	The authors commented that the significant increase in food consumption observed in the highest dose group without a corresponding effect on body weight indicated an effect on food utilization.
Conclusion remarks	The authors concluded that FD&C Yellow No. 5 was not developmentally toxic or teratogenic under the conditions of the study. The NOAEL's for maternal and fetal toxicity were greater than 1000 mg/kg bw/day.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.

References	Collins T., Black T.N., Brown L.H., and Bulhack P. (1990) Study of the teratogenic potential of FD & C Yellow No. 5 when given by gavage to rats. Fd. Chem. Toxic. Vol 28, pp 821-827.

4.5 REPRODUCTIVE TOXICITY

CAS Numerical	1934-21-0
Substance Name	C.I. Acid Yellow 23
Remarks for Substance	FD&C Yellow 5; 90% purity; 10% intermediates or volatile matter
Method/guideline	Lifetime Toxicity/Carcinogenicity study
Test Type	
GLP	Ambiguous
Year	1988
Species/Strain	Rats/Charles River CD
Sex	Male and Female
Route of administration	Oral-Diet
Duration of test	114 weeks
Doses/concentration levels	0, 0.1, 1.0, or 2.0% (original study) 0, 5.0% (high dose study)
Premating Exposure period for males	2 months
Premating Exposure period for females	2 months
Frequency of treatment	Daily
Control Group and treatment	Yes.
Remarks for test conditions	In the *in utero* phase, groups of rats (60/sex/group) were administered 0, 0, 0.1, 1.0 or 2.0% FD & C Yellow No. 5 in the diet daily for approximately 2 months prior to mating. In the high-dose study, 60/sex/group received 0 or 5% FD&C Yellow 5 for approximately 2 months prior to mating. The 3 controls groups received the basal diet only. A maximum of 2 rats/sex/litter were randomly selected for the chronic phase of the study. There were 70/sex/group at the initiation of the chronic phase and these offspring were exposed to the same dietary levels as their parents.

	Animals were housed individually and fed the test diet ad libitum. Clinical observations were recorded twice daily with at least 5 hours between observations. Detailed physical examinations and palpation for masses were performed weekly. Body weights and food consumption were determined weekly for the first fourteen weeks, bi-weekly for the next 12 weeks and every 4 weeks thereafter until the end of the study. The intake of the test substance was determined from body weight, food consumption and dietary concentration. Hematology tests, including hemoglobin, hematocrit, erythrocyte and total and differential leukocyte counts, and erythrocyte morphology, were conducted on ten randomly selected animals at months 3, 6, 12, 18 and 24 of the study. Necropsies were conducted on all animals dying prior to study termination, killed in a moribund condition or killed on schedule. Histological examinations were conducted on all animals from both control groups, the highest dose group (2.0 or 5.0%) from each study and also on 10 rats randomly selected from each group for an interim sacrifice at 12 months. Histology was also performed on any animal with gross lesions or masses. Tissues examined included adrenal glands, aorta, blood smear, brain, cecum, colon, duodenum, epididymus or uterus, esophagus, eyes, femur including marrow, tissue masses, gallbladder, heart, ileum, jejunum, duodenum, kidneys, liver, lungs and bronchi, mammary gland, nerves (sciatic), ovaries, lymph nodes, pancreas, parathyroids, pituitary gland, prostrate, rectum, skin, spleen, seminal vesicles, skeletal muscle, testes with epididymides, stomach, thymus, thyroid gland including parathyroid, trachea, urinary bladder, uterus.
NOAEL(NOEL)	5.0 % (Males: 2641 mg/kg/d and Females: 3348 mg/kg/day)
LOAEL(LOEL)	Not determined
Actual dose received by dose level and sex	Males: 48, 491, 984 or 2641 mg/kg/day Females: 58, 589, 1225 or 3348 mg/kg/d
Parental data and F1 as appropriate	In utero There were no compound-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or number of live and still-born pups. Slight decreases in body weight (4-5%) and slight increases in food consumption were noted in the F0 rats treated at dietary level of 5.0%. Two F0 female controls rats died during the in utero phase of the original study and one male and one female from the control and 5.0% group, respectively, died during the in utero phases of the high-dose study. There were no compound-related effects on pup survival.
Offspring toxicity F1 and F2	In the F1 generation, a yellow tint was reported at all intake levels above 0.1%. At the 1.0% dietary level, group mean body weights at termination for both sexes were lower than the control animals, but the difference was only statistically significant for the females. In the high dose study (5.0% dietary level), group mean body weights were significantly lower in both sexes at termination. Food consumption was similar for control and treated animals at the 0.01, 1 or 2% dietary levels,

	but was slightly higher at the 5% level in the high-dose study, although not statistically significant. Hematological, clinical chemistry and urinalysis parameters did not differ significantly from the controls. Necropsies at one year did not reveal any treatment-related gross or microscopic changes. At study termination, no treatment-related effects were reported on survival. No treatment-related changes were reported at gross necropsy. Histological evaluation revealed a variety of lesions, including neoplasms, present at similar incidences in control and treated animals. The authors considered the lesions to be spontaneous and not related to administration of the test material.
Appropriate statistical evaluations?	Yes, F-test, Anova
Remarks for results	The decrease in mean body weight at the 5.0% treatment level was not considered toxicologically significant give the non-nutritive character of FD & C Yellow No. 5.
Conclusion remarks	
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Borzelleca J. and Hallagan J. (1988a) A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (Tartazine) in rats. Fd Chem Toxic 26, 179-187.